N-Methyl-D-Aspartate Receptor (NMDA) Antagonists as Potential Pain Therapeutics

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Abstract: NMDA receptors are known to be involved in nociceptive transmission and pain processing. Many structurally diverse NMDA antagonists have been reported to have activity in both animal models and clinical models of neuropathic pain. Untoward side effects such as ataxia and sedation have severely limited the clinical uses of this class of potential therapeutics. However, antagonists at the glycine-site, NR2B sites and weak-binding channel blockers have demonstrated an improved side effect profile in animal models of pain. These types of compounds may hold potential promise for future pain therapies. This review covers reported pain data surrounding representative examples of NMDA antagonists and provides a current assessment of potential clinical utility.

INTRODUCTION

Based on considerable evidence, it has been recognized that N-methyl-D-aspartate (NMDA) receptors are partially responsible for the onset and maintenance of neuropathic pain [1]. This link has been established from *in vitro* pharmacology studies, behavioral models, animal models of neuropathic pain and clinical trial data. However, side effects such as ataxia and sedation have severely limited the clinical use of NMDA antagonists [2]. A deeper understanding of NMDA receptor pharmacology (*e.g.* identification of multiple antagonist binding sites and subtype specificity) has led to the second-generation antagonists (glycine-site, NR2B, weak affinity channel blockers) that may hold greater promise for future pain therapies with a more tolerable side effect profile [3]. The purpose of this review is to summarize the relevant *in vitro* pharmacological data, *in vivo* pain data and clinical trial-pain data from representative examples of the four classes of NMDA antagonists, which are: a) non-competitive channel blockers, b) competitive glutamate-site antagonists, c) competitive glycine-site antagonists and d) non-competitive antagonists of allosteric nature acting at sites linked to polyamine or ifenprodil action.

Glutamate is a critical excitatory amino acid that is important for nociceptive processing in the spinal cord [4]. Glutamate receptors can be divided into two families, ionotropic (ligand-gated ion channels) and metabotropic (G-protein coupled) [5]. Ionotropic receptors are further sub-divided into three classes: α-amino-3-hydroxy-5-methyl-4-isoxazole-proprionic acid receptors (AMPA), kainate receptors (KA) and NMDA receptors [6,7]. Among the ionotropic glutamate receptors, NMDA receptors are unique in that channel activation requires not only interaction with glutamate, but also another amino acid, glycine [8,9,10]. The glycine binding-site is distinct from the glutamate binding site, and glycine thus is a true co-agonist. Furthermore, modulatory ligands, like polyamines can alter the response of NMDA receptors to both glutamate and glycine [11-14].

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NMDA
N-methyl-D-aspartic acid

Glycine

Fig. (1). Excitatory amino acids acting at the NMDA receptor.

MOLECULAR STRUCTURE OF NMDA RECEPTORS

Functional NMDA receptors are heteromultimeric complexes likely to consist of several subunits [15,16]. Three distinct gene families have been identified that encode these subunits. The NR1 subunit is encoded by a single gene. However, because this gene can be spliced at three sites, the NR1 protein can exist as eight different isoforms [17]. The NR2 subunit is encoded by four different genes generating the NR2A, NR2B, NR2C and NR2D subunits, respectively [18, 19]. An NR3 subunit is also known and is proposed to play a regulatory role in development, perhaps in a protective sense [20]. The most common combination of these subunits in a functional NMDA receptor appears to be a complex of two NR1 subunits and two NR2 subunits [21].

Ligand sites of interaction are well-characterized with respect to receptor subtype. The glycine binding site is located on the NR1 subunit, whereas the glutamate site is located on the NR2 subunit [22-24]. It is also believed that polyamine-site antagonists such as ifenprodil bind at the NR2B subunit [25]. There is a reported high-affinity Zn^{2+} binding site located on the NR2A subunit that shares similarities to the ifenprodil site in the NR2B subunit [26]. All of the subtypes are heterogeneously distributed in brain and CNS [27, 28, 29]. The implications of subtype distribution are not yet fully understood. The past few years have witnessed huge advances in our understanding of the molecular structure of ionotropic glutamate receptors (iGluRs), including the NMDA receptor. These new insights have helped dramatically in the understanding of ligand interaction and it appears appropriate to briefly discuss them

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here. For further reading the reader is referred to a recent review on the topic [30].

The extracellular regions of all iGluR subunits comprise the extracellular N-terminus of approximately 500 residues and the region between the second and third transmembrane region. Inserted between these two extracellular regions is the pore-forming region consisting of two transmembrane regions separated by a hairpin-formed P-loop that forms the narrowest part of the actual ion channel (selectivity filter). A third transmembrane region and a large intracellular C-terminus follow the second extracellular region [31,32]. Interestingly, the two extracellular regions have similarity to periplasmic binding proteins (PBP) found in some bacteria [23, 33-35]. The most distal part of the N-terminus, comprising approximately the first 350 residues of iGluR subunits, has similarities to a PBP that in bacteria binds the amino–acids leucine, isoleucine, and valine [36]. In iGluRs this region was thus initially referred to as the LIVBP-like region. However, because the actual similarities between this region and the LIVBP of bacteria are weak, most authors prefer at present the more neutral label ATD (amino-terminal domain) for this region [37, 38]. The remainder of the N-terminus and the extracellular region between transmembrane 2 and 3 of all iGluR subunits has similarities to several PBP, with the strongest similarity to the bacterial glutamine binding protein [39]. In iGluR subunits this region is thus referred to as the GlnBP-like region. Note that earlier literature referred to this region as the LAOBP-like region, from the bacterial lysine, arginine, ornithine binding protein [40].

Agonists bind to iGluRs at the GlnBP-like region [30, 31]. At NMDA receptors, glutamate binds to the GlnBP-like region of the NR2 subunits, and the co-agonist glycine binds to the GlnBP-like region of NR1 [23, 24]. Opening of NMDA receptor ion channels is likely to require occupancy of all GlnBP-like regions of the receptor by the respective agonist, (*i.e.* binding of two molecules of glycine and glutamate each to one NMDA channel) [41]. Competitive antagonists interfere with binding of glycine to the GlnBP-like region of NR1 or with binding of glutamate to the GlnBP-like region of NR2.

Ligand binding to the ATD region does not activate iGluRs, but has modulatory, allosteric roles [37, 38]. The ATD of NR2A has been shown to influence NMDA receptor desensitization [42, 43], and this effect seems, at least partially, to be mediated by high-affinity binding of Zn^{2+} to the ATD of NR2A [26]. Likewise, binding of ifenprodil to the ATD of NR2B inhibits activity of NMDA receptors containing this subunit [25]. No ligands have yet been identified that bind to the ATD of NR2C or NR2D subunits, but such ligands may hold potential for subtype-specific modulation of NMDA receptors.

From crystallization studies it was known that in bacteria the binding proteins form a "clam-shell" like structure, that can exist in an "open" and "closed" configuration, with the ligands stabilizing the closed configuration [35, 36, 39, 40]. A similar scenario was thus envisioned for ligand action at iGluRs [23, 33]. Crystallization studies of the isolated GlnBP-like regions of iGluRs [44], including the GlnBP-like region of the NR1 subunit [45, 46], have largely confirmed this picture. Interestingly, crystals of the GlnBP-like region of NR1 grown in the presence of the agonist glycine or several partial agonists result in a "closed" clam-shell structure, whereas crystals grown in the presence of a glycine site antagonist reveal an "open" clam-shell structure [46]. These recent studies are likely to herald a new era of computational approaches to the development of NMDA receptor antagonists and modulators.

PHYSIOLOGICAL ROLE OF NMDA RECEPTORS

NMDA receptors play a key role in integrating and transforming fast synaptic signaling into slower dynamic modifications at the intracellular level. This integratory function is in large part achieved by two biophysical properties of open NMDA receptors: voltage-dependent block by extracellular magnesium ions, and permeability to

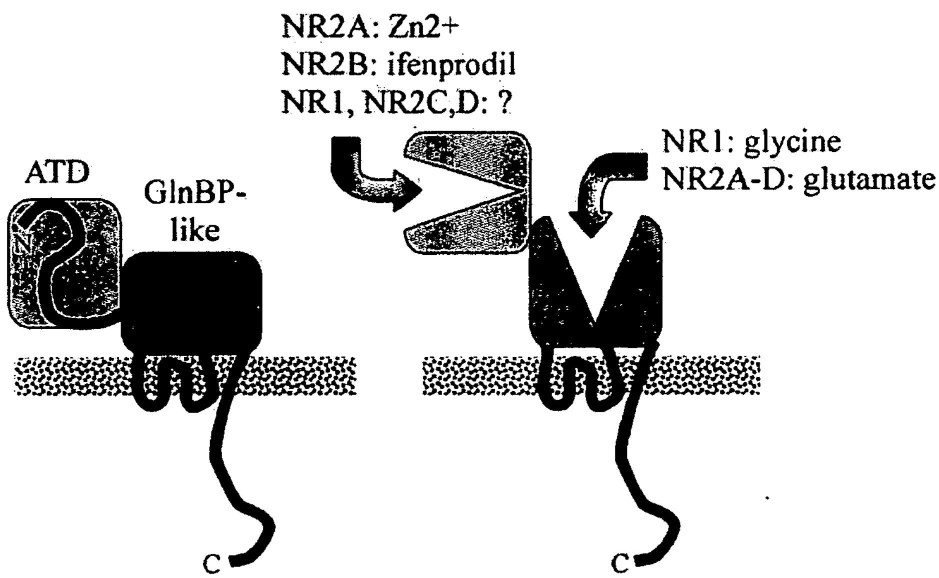


Fig. (2). Schematic representation of NMDA receptor.

calcium ions. At membrane potentials that correspond to the membrane potential of most neurons at rest (below –50 mV), extracellular magnesium ions block NMDA receptors, even when the receptor is activated by both agonists, glycine and glutamate [47]. However, due to the charged nature of Mg^{2+}, this block is voltage dependent and is readily reversed upon depolarization of the membrane. In the case of spinal nociceptive processing, depolarization is achieved by the same molecular signal that activates NMDA receptors: glutamate release from A- and C-fibers terminating in the spinal cord will open AMPA and KA channels and thereby depolarize the spinal cord neurons sufficiently to induce relief of Mg^{2+}-block of the co-localized NMDA receptors [48]. Because open NMDA channels are permeable not only to monovalent cations like Na^+ and K^+, but also to Ca^{2+}, this release from extracellular magnesium block will result in a transient and spatially restricted elevation of intracellular calcium levels. This in turn will trigger activation of calcium-dependent proteins localized within the affected area, and subsequent activation of other cellular processes, that can ultimately lead to cell death in the extreme of over-excitation [49]. As part of the regulation of glutamate receptors, released glutamate is removed from extracellular space by a glutamate transport system, which then allows the NMDA receptors to return to a resting state [50].

NMDA RECEPTORS AND CENTRAL SENSITIZATION

Chronic neuropathic pain is a result of pathological alterations in the central nervous system that persist long after the painful stimuli has been removed [51-53]. One of these types of pathological alterations is termed central sensitization and is a result of hyperexcitability of dorsal horn neurons [54]. Sensitization can occur after repeated stimuli of peripheral inputs, chemical stimulation or central pathological events [55]. Another related circuitry change is a specific phenomenon known as "wind-up", caused by repetitive C-fiber activation that results in abnormal firing of the dorsal horn neurons [56]. It is also hypothesized that spinal cord injuries result in changes of the circuitry and lead to central sensitization [57]. Furthermore, it is believed that central sensitization may underlie the neuropathological pain conditions of hyperalgesia (exaggerated nociceptive response to noxious stimuli) and allodynia (nociceptive responses to innocuous stimuli) [58].

It has been established that NMDA receptors are involved in central sensitization in the dorsal horn of spinal cord [59, 60, 61]. As an example, it has been demonstrated that NMDA antagonists can both block and reverse central sensitization [59]. Similarly, the phenomena of wind-up has been linked to NMDA receptors [62, 63]. For example, ketamine [60], MK-801 [64] and memantine [64] have all been reported to inhibit wind-up on dorsal horn neurons.

EVIDENCE OF GLUTAMATE AND GLUTAMATE RECEPTOR REGULATION IN NOCICEPTIVE PROCESSING

During nociceptive events, the number and subunit composition of glutamate receptors are altered from the resting state. For example, following the administration of Freund's complete adjuvant (FCA), the numbers of peripheral glutamate receptors in primary afferent neurons were increased following immunochemical staining [65]. It has likewise been observed that in the formalin pain model, differences in NMDA receptor expression are noticed. After administration of formalin (in the formalin pain model), the levels of NR2A expression are increased in the spinal cord while NR2C levels are decreased [66]. In a different study, spinal nerve lesion resulted in a decrease of NR2A receptors in the dorsal horn [67]. Another study has also implicated the NR1 subunit in central sensitization. Conditional knock-out mice of the NR1 NMDA subunit in the lumbar spinal cord had diminished nociception in the formalin pain model, but did not have alterations in normal pain thresholds to cold, heat or mechanical stimulation [68].

Along with changes in NMDA receptor populations, concentrations of glutamate are like-wise altered in animal pain models. In one study the concentration of peripheral glutamate increased in the ipsilateral paw, but not the contralateral paw following formalin injection [69]. In another study, concentrations of glutamate and aspartate increased in the ipsilateral side of the dorsal horn in a rat CCI model of neuropathic pain. Treatment of MK-801 led to a suppression of this excitatory amino acid increase [70]. Consistent with the link between elevated levels of glutamate in certain pain models, it has been observed that intrathecal L-glutamate can cause allodynia by itself [71].

NMDA ANTAGONISTS IN MODELS OF PAIN

The link between NMDA receptors and neuropathic pain has been well validated in animal models of pain. One early report of an NMDA antagonist in pain model was that of APV, (intrathecal administration), however these studies demonstrated side effects which complicated the analysis [72]. Since then, all of the various classes of NMDA antagonists have been studied in a variety of pain models and are summarized in this review.

A very common pain model that has been used to profile a wide-variety of structurally diverse NMDA antagonists is the formalin pain model [73, 74]. This model has two distinct phases of nociception that are believed to represent peripheral and centrally mediated pain. The early phase (EP) (transient) is thought to be a response to the peripheral nociception [75], whereas the late-phase (LP) is hypothesized to be the result of changes in the CNS function including central sensitization of dorsal horn neurons [76]. Formalin also causes excitation of C-fibers in a biphasic manner that is consistent with EP and LP observations in rat models of formalin induced pain [62]. Some have suggested that this model is similar to post-operative pain [77].

Another well studied pain model with respect to NMDA antagonists is the nerve injury or ligation model, of which several varieties are known, including the chronic constrictive injury model (CCI model) [78], the spinal nerve ligation model (Chung model) [79] and the partial sciatic nerve injury model [80]. Other pain models are included in this report, even though their link to human neuropathic pain or NMDA receptor activity has not been as well characterized. Studies in acute pain models (*e.g.* tail flick test) have also been included in some circumstances in order to provide the reader some comparison to the various models

used with NMDA antagonists. It is generally accepted that most NMDA antagonists do not work in these models [81], although there are some exceptions in the tables below. It has been reported that NMDA antagonist side effects can sometimes confound the results of certain acute pain models [81], and thus these results must be taken into the broader context of knowledge about NMDA antagonists and motor/CNS function side-effect profiles.

There have also been many reported studies in both acute pain models and chronic pain models on the effect of NMDA antagonists to potentiate low-doses of other classes of pain drugs such as opiates [82, 83]. It is not the purpose of this review to cover this large topic, however, some selected examples are discussed where direct comparisons could be made either to the compound or compounds of similar type in a relevant pain model.

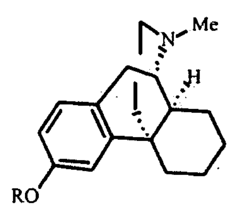
The underlying hypothesis and potential value of NMDA antagonists rests on the potential for blocking pathological pain mechanisms without severe impact to the normal physiological pain processes. Evidence for this hypothesis was largely driven by electrophysiological data indicating that NMDA antagonists can block wind-up without alteration of basal neuronal firing [84]. This hypothesis was reinforced by behavioral data supporting the fact that NMDA antagonists in general do not alter basal paw withdrawal latencies in the contralateral paw in models of neuropathic pain [85]. In this respect NMDA antagonists may be seen as "targeted therapy" and may have advantages over drugs which attenuate normal physiological pain in a broad sense.

The following sections of this review contain summaries of *in vitro* pharmacology, pain-related behavioral models, animal models of pain as well as human clinical trial results. Although this covers a large majority of known NMDA antagonists, it is not meant to be a comprehensive collection of all known studies. References and compounds were selected so as to provide both a breadth of diversity of compounds, a comparison to the most well-studied compounds, and a historical perspective of the development of individual classes of antagonists. The sections are subdivided into the four classes of antagonists.

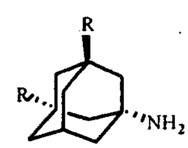
NON-COMPETITIVE ANTAGONISTS: CHANNEL BLOCKERS

The NMDA channel is characterized by a high affinity to PCP with a reported K_d value for PCP of 0.027 μM in rat brain synaptic membranes [86]. PCP is not considered a candidate for neuropathic pain treatments due to an obvious liability of psychotomimetic side effects and abuse potential. Therefore, the challenge in finding channel blockers with therapeutic utility is to identify compounds which lack PCP-like pharmacology. Chemical structures of the compounds addressed in this section are shown in Figure 3. Compounds reported to be channel blockers that have been tested in pain models are then summarized in Table 1.

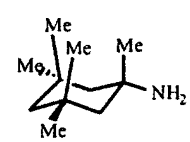
Dextromethorphan and related analogs are similar in structure to the opiods, but differ in that they possess the dextrorotary configuration. This stereochemical change results in a loss of significant opiod activities [87]. Clinically, dextromethorphan is currently used as an anti-tussive. Dextromethorphan shows modest selectivity for NR1A/NR2A over NR1A/NR2B (approx. 4-fold) [88]. A related compound, dextrorphan, is a more potent metabolic side product of dextromethorphan. For a detailed discussion of dextrorphan binding see Franklin and Murray [89].



Dextromethorphan, R = Me
Dextrorphan, R = H



Memantine, R = Me
Amantadine, R = H



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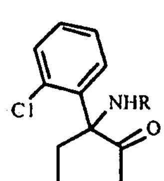
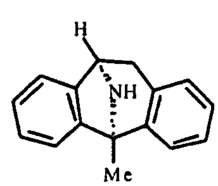


Ketamine, R = Me
Norketamine, R = H



MK-801
Dizocilpine

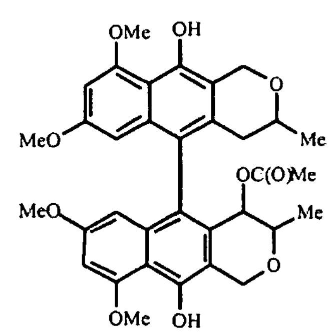


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Fig. (3). Non-Competitive NMDA Antagonists.

Table 1. Pain Data Associated with Non-Competitive NMDA Receptor Antagonists

Antagonist/*In Vitro* activity	Pain Models	Admin.[a]	Species[a]	Result[b]	Lack Side Effect[b]	Ref.
Dextromethorphan IC_{50} = 2.5 µM [86][c]	Formalin	i.th.	r	(+/-)		[107]
	Chung Model	i.th.	r	(+)	(+)[d]	[107]
	Mechanical allodynia, spinal injury	i.p.	r	(+)	(+)	[108]
	Opiate enhancement		h	(-)		[109]
	Writhing, NSAID potentiation	i.p.	m	(+)		[110]
	Diabetic neuropathy	p.o.	h	(+)	(-)	[111]
	Postherpatic neuralgia	p.o.	h	(-)		[111]
	Oral surgery, post-operative pain	p.o.	h	(+)[e]	(-)[f]	[112]
	Heat-capsaicin model, volunteers	i.v.	h	(+)	(-)[g]	[113]
Dextrorphan IC_{50} = 0.68 µM [86][c]	Chung Model	i.th.	r	(+)	(+)[d]	[107]
	Formalin, LP	i.th.	r	(+/-)		[107]
	Formalin, LP	i. pl.	r	(+/-)[h]		[114]
	Thermal hyperalgesia, CCI	i.p.	r	(+)		[115]
	Mechanical hyperalgesia, CCI	i.p.	r	(-)		[115]
	Tail-flick- opiate potentiation, spinal injury	s.c.	r	(+)	(-)	[116]
Memantine K_i = 2.45 µM [97][i]	Chung Model	i.th.	r	(+)	(+)[d]	[107]
	Formalin, LP	i.th.	r	(+)	(+)[d]	[107]
	Formalin, EP	s.c.	m	(+)	(-)	[74]
	Formalin, LP	s.c.	m	(+)	(-)	[74]
	Formalin, facial pain, EP	i.p.	r	(+/-)		[117]
	Formalin, facial pain, LP	i.p.	r	(+)	(+)	[117]
	Formalin, LP	i. pl.	r	(+/-)		[114]
	Thermal hyperalgesia, CCI	i.p.	r	(+)	(+)	[118]
	Thermal hyperalgesia, carrageenan	s.c.	r	(+)	(+)	[119]
	Visceral pain, uterer distension	i.v.	r	(+)		[120]
	Thermal hypersensitivity, PGE2	i.p.	r	(-)		[121]
	Toe-pinch	i.v.	r	(+)[j]		[120]
	Writhing, NSAID potentiation	i.p.	m	(+)		[110]
	Diabetic neuropathy	p.o.	h	(-)		[111]
	Postherpatic neuralgia	p.o.	h	(-)		[111]
	Post-operative pain, amputations, nerve injuries		h	(-)		[122]
Amantadine K_i = 25.87 µM [97][i]	Surgical neuropathic pain, cancer patients	i.v.	h	(+)	(+)	[123]
	Neuropathic pain	i.v.	h	(+)[k]		[124]
	Diabetic neuropathy	i.v.	h	(+)		[125]

(Table 1) Contd....

Antagonist/*In Vitro* activity	Pain Models	Admin.[a]	Species[a]	Result[b]	Lack Side Effect[b]	Ref.
MRZ 2/579 K_i = 1.47 µM [97][l]	Thermal hyperalgesia, carrageenan		r	(+)	(+)[l]	[96]
Ketamine IC_{50} = 0.46 µM [129][o]	Thermal hyperalgesia.	i.th.	r	(+)	(+)	[126]
	Pressure, opiate potentiation	s.c.	r	(+)		[127]
	Carrageenan, opiate potentiation	s.c.	r	(+)		[128]
	Tail flick	i.th.	m	(-)		[129]
	Chung Model	i.th.	r	(-)		[107]
	Thermal hyperalgesia, carrageenan	i.th.	r	(+)	(+)	[85]
	Formalin, EP	s.c.	m	(+)	(-)	[74]
	Formalin, LP	s.c.	m	(+)	(-)	[74]
	Formalin, LP	i. pl.	r	(+/-)		[114]
	Formalin, LP	p.o.	r	(+)	(+)[l]	[99]
	Mechanical allodynia, transgenic[j]	i.p.	m	(+)		[130]
	Visceral pain, uterer distension	i.v.	r	(+)		[120]
	Toe-pinch	i.v.	r	(-)		[120]
	Writhing test, NSAID potentiation	i.p.	m	(+)		[110]
	Thermal hypersensitivity, PGE2	i.p.	r	(-)		[121]
	Fibromyalgia patients[m]	i.v.	h	(+)		[131]
	Secondary hyperalgesia/ "wind-up" like pain	i.v.	h	(+)	(-)	[132-134]
	Neuropathic pain	i.v.	h	(+)	(-)	[135]
	Heterogeneous painful neuropathies	i.v.	h	(+)	(-)	[104]
	Stump /phantom limb-pain -amputees	i.v.	h	(+/-)	(-)	[136]
	Experimental ischemic pain /	i.v.	h	(+)	(+)	[137]
	Postoperative pain, oral surgery	i.v.	h	(+)	(+)	[137]
MK-801 IC_{50} = 0.015 µM [129][o]	Tail flick test	i.th.	m	(-)		[129]
	Thermal hyperalgesia, CCI	i.th.	r	(+)	(+)	[126]
	Thermal hyperalgesia, CCI	i.p.	r	(+)		[138, 139]
	Self-mutilation, nerve injury	i.th.	r	(+)		[140]
	Chung model	i.th.	r	(-)		[107]
	Formalin, LP	i.th.	r	(+)	(+)[d]	[107]
	Formalin, EP	s.c.	m	(+)	(-)[d]	[74]
	Formalin, LP	s.c.	m	(+)	(-)[d]	[74]
	Mechanical allodynia, nerve injury	i.p.	r	(+)	(-)	[141]
	Mechanical hyperalgesia, carrageenan	i.p.	r	(+)	(-)	[141]
	Thermal hyperalgesia, carrageenan	i.th.	r	(+)	(+)	[85]

(Table 1) Contd....

Antagonist/*In Vitro* activity	Pain Models	Admin.[a]	Species[a]	Result[b]	Lack Side Effect[b]	Ref.
	Thermal hyperalgesia, nerve injury	i.th.	r	(+)	(+)	[142]
	Mechanical allodynia, spinal cord injury	i.p.	r	(+)	(-)	[108]
	Mechanical hyperalgesia, post-operative pain	i. th.	r	(-)		[143]
	Mechanical hyperalgesia (periph.), FCA	i. pl.	r	(+)		[144]
	Allodynia, opiate induced	s.c.	r	(+)		[145]
	Thermal hypersensitivity, PGE2	i.p.	r	(-)		[121]
Mg^{2+}	CCI	i.p	r	(+)	(+)	[146]
	Formalin, LP	i.th.	r	(+)	(+)	[147]
	Heterogeneous painful neuropathies, spontaneous pain, allodynia	i.v.	h	(-)		[104]
ES-242-1 IC_{50} = 0.116 μM [105,106][o]	Formalin, EP	i.th.	r	(+)		[148]
	Formalin, LP	i.th.	r	(+)		[148]

[a] Route of administration and species tested: i.th. = intrathecal, i.p. = intraperitoneal, i.m. = intramuscular, i. pl. = intraplantar, s.c. = subcutaneously, i.v. = intravenously, p.o. = oral,; r = rat, m = mouse, h = human. [b] Result : (+) = postive result, (-) = negative result, (+/-) = limited efficacy or incomplete attentuation. Side effect separation: (+) = no noticeable effects in assay, (-) = noticeable effects, no value in column indicates that the side effect profile was not reported. [c] Versus [^{3}H] PCP. [d] Separation observed in separate motor side effect assay. [e] 48 hours post surgery, but not at 6 hours. [f] Adverse effects seen above placebo. [g] Drowsiness reported after infusion. [h] Lifting behaviors and righting reflexes were attenuated, but not flinching. [i] Versus [^{3}H] MK-801. [j] Authors claim positive effect may be due to nonspecific cardiovascular effects. [k] Tested in only three patients. [l] Positive (30 mg/kg), but side effects evident at 60 mg/kg p.o. [m] Muscle pain, temporal summation, and referred pain. [n] Versus [^{3}H] TCP. [o] Rat brain receptors in Xenopus oocytes.

Memantine is a clinically available (Parkinson's disease, and more recently Alzheimer's disease) NMDA antagonist with a low side effect profile [90, 91]. It is reported to have no selectivity for NR1A/NR2A over NR1A/NR2B [88]. Memantine is considered unique among the channel-blockers because it displays fast blocking kinetics and possesses a strong voltage-dependence of the block. It is surmised that these properties may contribute to the lack of PCP-like side effects [92, 93]. A recent report has coined memantine as being part of a class of "pathologically activated therapeutics"(PAT), thus describing its relatively good side-effect profile under non-stress conditions [94].

In electrophysiology studies, memantine (*s.c.* admin.) reduced neuronal wind-up in a Chung model with little effect on sham-operated rats (in contrast to MK-801 and ketamine which reduced wind-up in both animal models) [91]. It has demonstrated a reduced activity on spinal cord neurons in a rat arthritis model in response to noxious and innocuous stimuli (*i.v.* admin.) [95]. With respect to side effect profiles, it does not show activity in pre-pulse inhibition of the acoustic startle reflex (PPI), a model of psychotomimetic side effects. This result contrasts with MK-801 and PCP which do show adverse events in this model [96]. Other related analogs to memantine are amantadine and MRZ 2/579. Amantadine is clinically used as an anti-viral compound and has very weak channel blocking properties. MRZ 2/579, which is similar in structure to memantine, also displays fast blocking kinetics (K_{on} = 10.67 ± 0.09 x 10^4 M^{-1} s^{-1}, K_{off} 0.199 +/- 0.02 s^{-1}, K_d = K_{off}/K_{on} = 1.87 μM.) [97]. Again, the authors argue that low-affinity channel blockers such as memantine and MRZ 2/579 have a clear advantage over high affinity channel blockers such as MK-801 with respect to an improved side effect profile. Alternatively, this could be due to other factors including blocking kinetics, voltage dependence or subtype-selectivity [96].

Ketamine is another member of the non-competitive NMDA channel blocking antagonists, but also possesses activity at other receptors including opiod, monoaminergic, muscarinic, and voltage-sensitive Ca^{2+} channels [98]. Intravenous administration to rats during the second phase of formalin prevented dorsal horn nociceptive neuron firing in a dose-related manner, with only a small effect in the first phase [73]. Ketamine can be rapidly metabolized to norketamine, which has equipotent activities to ketamine itself. Thus, the authors conclude that norketamine could be responsible for part of the nociception observed with ketamine [99].

Ketamine is generally not used in the clinic because it induces a trance-like cataleptic state [100]. A more comprehensive report on ketamine clinical trial data has been published, and the conclusion from the data (53 clinical trials) is that the role of ketamine in perioperative pain is unclear [101].

MK-801 is a potent channel-blocker with no selectivity for NR1A/NR2A over NR1A/NR2B [88]. When MK-801 was iontophoretically administered to CCI rats with thermal and mechanical stimulated hyperalgesia, it blocked noxious

evoked response in WDR neurons, but not baseline hyperactivity. It is suggested by the authors that the baseline hyperactivity is likely related to spontaneous pain [102]. Administration (*i.v.*) to rats during the second phase of the formalin pain assay prevented dorsal horn nociceptive neuron firing in a dose-related manner, with only a small effect in the first phase. Pre-treatment before formalin also gave dose-related inhibition with little effect on the first phase [73]. Similarly when dosed intravenously to rats, MK-801 blocked noxious-stimuli evoked activity in CCI rats in ventroposterolateral neurons as measured by electrophysiology. However, it also blocked this activity in normal rats in contrast to the glycine-site antagonist GV-196771A [103]. Pain studies for MK-801 are summarized in Table 1.

Since magnesium serves as an endogenous channel-blocker, magnesium salts have been studied as potential pain therapeutics. At least one clinical trial has been reported, however, magnesium salts were not efficacious in this study [104].

Finally, a microbial natural product, ES-242-1, is reported to interact with NMDA receptors, predominately as a channel blocker. However, ES-242-1 also has competitive activity at the glutamate binding site (IC_{50} = 1.1 μM *vs*. [^{3}H] CPP) [105, 106]. The structure appears to be quite unique in that it does not possess a basic amine. Very limited studies have been done in pain studies with this compound, however these results are shown in Table 1.

COMPETITIVE GLUTAMATE SITE ANTAGONISTS

Most of the glutamate-competitive antagonists that are reported in Figure 4 belong to the phosphono-amino-acid class of compounds. These compounds were originally developed for treatment of stroke and stroke related diseases, but have so far not proved clinically useful for these applications. One reason for this is a poor therapeutic margin and the large doses required for optimal brain penetration [149]. Subsequent studies in animal pain models have demonstrated activity, with however, the expected side effects being observed. Although there are some exceptions when comparing different pain models, most of these compounds have motor side effects with a narrow therapeutic window. In contrast to the channel blockers (non-competitive), many of the glutamate-competitive antagonists are active in both early phase and late phase formalin. In some cases these compounds are also active in tonic (phasic) pain models. In the various studies reported these compounds are typically administered intrathecally, suggesting that blood-brain barrier (BBB) penetration may be difficult for some of these compounds. However, some examples of systemic administration are included below. Two naturally occurring peptides are included in this review Con-G and Con-T. Although these may not represent small molecule therapeutics, it is useful to highlight the structural diversity in this class of antagonists.

CPP is a potent and competitive antagonist. Intrathecal administration to rats prevented dose-related dorsal horn nociceptive neuron firing in the second phase of formalin assay with only a small observable effect in the first phase [73]. Very limited studies have been reported in pain assays, most of which resulted in the observation of motor side effects at therapeutic doses (Table 2). Similar to CPP, 2-amino-5-phosphonovalerate (APV or AP-5) dose-dependently prevented dorsal horn nociceptive neuron firing in the second phase of the formalin assay, with only a small effect in the first phase [73]. APV has also been reported to reduce electrically stimulated wind-up in rats [162]. Injection into the hippocampal dentate gyrus, before and after formalin first phase, led to reduction of nociceptive behaviors. This result led the authors to conclude that pain related behaviors associated with the NMDA receptor could be involved in the hippocampal region [163]. When APV was co-administered with glycine an increase in the antinociceptive activity across a range of doses was observed in the mouse formalin model. The effect was reversed with the addition of 7-CKA [164]. There was no synergistic effect with morphine in thermal hot plate test (*i.th.* administration, rats) [165].

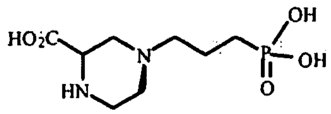
Conformationally restricted analogs of APV, such as CGS-19755 (Selfotel), MDL 100-925, LY235959, CGP 37849 and CGP 39551 have also been studied in pain models. The results are reported in Table 2. It may be expected that these compounds suffer from poor brain penetration due to the charged nature. This has been surmised for LY235959 which was less potent when administered subcutaneously as compared to an intrathecal route [158].

Perzinfotel (EAA-090) is also a high-affinity competitive antagonist for glutamate. The compound demonstrated a superior side effect profile over other NMDA antagonists of similar kind in PGE2 and capsaicin pain models. Of interest is the fact that some NMDA antagonists (ketamine, memantine, ifenprodil and others) did not show activity in this model, thus perhaps indicating a novel mechanism of action for perzinfotel [121].

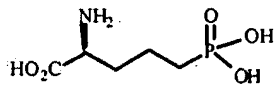
Conantokin G (CGX-1007 or Con-G) is a 17 amino-acid peptide isolated from cone snail venom of the genus *Conus*, and is reported to selectively bind to the NR2B subunit [161]. It has an IC_{50} of 0.48 μM on the NR2B-component of NMDA-evoked currents from cultured cortical neurons. No response was seen above 10 μM for the other subtypes [161]. Conantokin G exists in a helical form that is stabilized by the presence of divalent cations such as Ca^{2+} and Zn^{2+} [166, 167]. However, there does not appear to be a clear relationship between this property and the observed binding activity in the absence or presence of these metals [166, 167]. The mechanism of action of the conantokins has been challenging to understand. Although it is likely that these are competitive antagonists [166, 167, 168] others have suggested a role in the allosteric polyamine modulatory site [169, 170]. For a recent review the reader is referred to Castellino [171]. Conantokin T is an analog of conantokin G. This compound binds to both NR2A and NR2B [161, 171].

COMPETITIVE GLYCINE SITE ANTAGONISTS

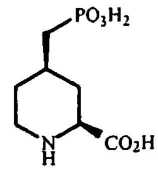
As discussed in the introduction, occupancy of the glycine site is required for activation of NMDA receptors. Among the lines of evidence in this regard is that glycine has been reported to enhance the affinity of MK-801 in washed rat cortical membranes [172]. Further evidence is gained by studies that have demonstrated an enhancement of NMDA-mediated post-synaptic potentials in neocortical slices



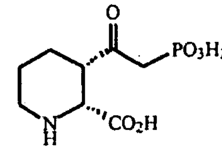
(±)CPP



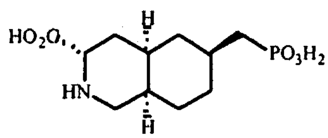
APV, AP5



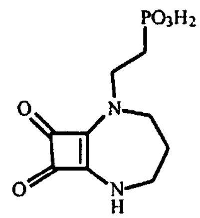
CGS 19755
Selfotel



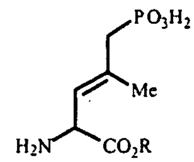
MDL 100,925



LY 235959



Perzinfotel = EAA-090



CGP 37849, R = H
CGP 39551, R = Me

NH2-GEγγLQγNQγLIRγKSN-CONH2

Conantokin G

γ = γ-carboxyglutamic acid

NH2-GEγγYQKMLγNLRγAEVKKNA-CONH2

Conantokin T

Fig. (4). Competitive NMDA Antagonists

Table 2. Pain Data Associated with Competitive Glutamate Site Antagonists

Antagonist/*In Vitro* activity	Pain Models	Admin.[a]	Species[a]	Result[b]	Lack Side Effect[b]	Ref.
CPP IC_{50} = 0.32 μM [150][c]	Thermal hyperalgesia, carrageenan	i.th.	r	(+)	(+)	[85]
	Formalin, LP	s.c.	m	(+)	(-)	[74]
	Tail flick	i. th.	r	(+)	(+)[d]	[152]
	Hot plate	i. th.	r	(+)	(+)[d]	[152]
	Formalin, EP	i. th.	r	(+)	(+)[d]	[152]
	Formalin, LP	i. th.	r	(+)	(+)[d]	[152]
	Thermal hypersensitivity, PGE2	i.p.	r	(+)		[121]
APV K_i = 0.35 μM [153][e]	Formalin, LP	i.th.	r	(+)	(-)	[151]
	Mechanical allodynia, spinal injury	i.th.	r	(+)	(+)	[57]
	Thermal allodynia spinal injury	i.th.	r	(-)		[57]
	Chung model	i.th.	r	(+)	(+)[f]	[107]
	Formalin, LP	i.th.	r	(+)	(+)[f]	[107]
	Thermal hyperalgesia, CCI	i.th.	r	(+)	(+)	[126]
	Self-mutilation, nerve injury	i.th.	r	(+)		[140]
	Mechanical hyperalgesia, incision	i.th.	r	(-)		[143]
	Thermal hyperalgesia, nerve injury	i.th.	r	(-)		[142]

(Table 2) Contd....

Antagonist/*In Vitro* activity	Pain Models	Admin.[a]	Species[a]	Result[b]	Lack Side Effect[b]	Ref.
	Thermal hyperalgesia, carrageenan	i.th.	r	(+)	(+)	[85]
	Muscle hyperalgesia, FCA	i.m.	r	(+)		[154]
CGS-19755 K_i =0.040 μM [155][e]	Thermal hypersensitivity, PGE2	i.p.	r	(-)		[121]
	Formalin, EP	s.c.	r	(-)		[156]
	Formalin, EP	s.c.	m	(+)	(-)[f]	[74]
	Formalin, LP	s.c.	m	(+)	(-)[f]	[74]
	Formalin, LP	s.c.	r	(+)	(+)[d]	[156]
	Mechanical allodynia, spinal cord injury	i.p.	r	(+)	(-)	[108]
MDL 100,925 K_i = 0.064 μM [155][e]	Hot plate	i.th.	m	(+)		[155]
	Formalin, EP	i. th.	m	(+)		[155]
	Formalin, LP	i. th.	m	(+)		[155]
LY235959 K_i = 0.025 μM [157][e]	Thermal hyperalgesia, NMDA	i. th.	r	(+)	(+)	[158]
	Formalin, LP	i. th.	r	(+)	(+)	[158]
	Formalin, LP	s. c.	r	(+)	(-)	[158]
CGP 37849 IC_{50} = 0.025 μM [150][c]	Formalin, EP	s.c.	m	(+)	(-)[f]	[74]
	Formalin, LP	s.c.	m	(+)	(-)[f]	[74]
	Tail-flick, heat or pressure	s.c.	m	(-)		[74]
CGP 39551 IC_{50} = 0.025 μM [150][c]	Formalin, EP	s.c.	m	(+)	(-)[f]	[74]
	Formalin, LP	s.c.	m	(+)	(-)[f]	[74]
	Tail-flick, heat or pressure	s.c.	m	(-)		[74]
Perzinfotel (EAA-090): IC50= 0.030 μM [159][h]	Thermal hypersensitivity, PGE2	i.p.	r	(+)		[121]
	Thermal hypersensitivity, PGE2	p.o.	r	(+)		[121]
	Thermal hypersensitivity, capsacin	i. p.	r	(+)		[121]
	Thermal hypersensitivity, capsacin	p.o.	r	(+)		[121]
Conantokin G IC_{50}=0.48 μM (electrophys) [161][i]	Formalin, EP	i. th.	m	(-)		[160]
	Formalin, LP	i. th.	m	(+)	(+)	[160]
	Thermal allodynia, nerve injury	i. th.	m	(+)		[160]
	Mechanical allodynia, nerve injury	i. th.	m	(+)		[160]
	Thermal allodynia, FCA	i. th.	m	(+)		[160]
	Mechanical allodynia, FCA	i. th.	m	(+)		[160]
Conantokin T	Formalin, EP	i. th.	m	(-)		[160]
	Formalin, LP	i. th.	m	(+)	(+)	[160]

[a] Route of administration and species tested: i.th. = intrathecal, i.p. = intraperitoneal, i.m. = intramuscular, i. pl. = intraplantar, s.c. = subcutaneously, i.v. = intravenously, p.o. = oral,; r = rat, m = mouse, h = human. [b] Result : (+) = postive result, (-) = negative result, (+/-) = limited efficacy or incomplete attentuation. Side effect separation (+) = no noticeable effects in assay, (-) = noticeable effects, no value indicates that side effect profile was not reported. [c] Versus [^{3}H] L-glutamate. [d] Narrow window versus side effects. [e] Versus [^{3}H] CGS 19755. [f] Side effects seen at higher doses. [g] Therapeutic doses tested in motor function assay. [h] Versus [^{3}H] CPP. [i] Determined in NMDA evoked currents in competition with CPP and other classes of NMDA antagonists.

following glycine application [173]. It is generally thought that glycine site antagonists may possess a better side effect profile over competitive antagonists and channel blockers [174].

Similar to competitive antagonists at the glutamate site, one difficult challenge surrounding glycine-site antagonists has been to identify compounds with high levels of brain penetration. This is most likely due to high plasma protein binding [175] and perhaps the charged nature of these compounds at physiological pH ranges. Interestingly, glycine itself has been shown to prevent mechanical hyperalgesia in rat models of neuropathic pain [176]. This would seem to contradict the theory that glycine-site antagonists are useful in neuropathic pain. However, these effects are proposed to be due to activation of the inhibitory glycine receptors (strychnine sensitive), which would decrease excitability of spinal neurons. A summary of known pain activities of glycine-site antagonists is described below. Some allosteric partial agonists are known, such as D-serine and D-cycloserine, and are reported in this review since they have been studied in pain models. Structures of these compounds are illustrated in Figure **5** and Figure **6**.

The early studies on indole carboxylic acids led to the development of some very potent glycine-site antagonists. The two most advanced indole carboxylic acids MDL 29,951 and GV196771A are reported in Table **3**. Dosed intravenously to rats, GV196771A blocked noxious-stimuli evoked activity in CCI rats in ventroposterolateral neurons as measured by electrophysiology. It did not alter this activity in normal rats, in contrast to MK-801 which blocked the activity in both sets of animals. The authors suggest that these compounds may block nociceptive signals in the thalamus [103]. GV196771A has also been reported to reduce the morphine tolerance in early phase and late phase formalin [198].

Another class of compounds, the quinoxaline-2,3-diones, have also been exploited as glycine-site antagonists. DCQX (6,7-dichloroquinoxaline-2,3-dione) is one of the first generation quinoxaline-2,3-diones, and has a reported IC_{50} *vs* [^{3}H] DPCQ of 0.13 μM [183]. It is important to note that some of these compounds do show activity against non-NMDA receptors (DCQX K_b = 4.8 μM in non-NMDA receptors, expressed receptors in oocytes [180]). Another example is ACEA-1011 with a reported affinity towards NMDA receptors expressed in Xenopus oocytes between 0.4 to 0.8 μM, while AMPA receptor binding activity was 8 μM in the same study [182].

While ACEA-1021 (Licostinel) is a much more potent analog in this series, it also possesses activity in non-NMDA receptors. ACEA-1021 has a reported affinity of K_b = 0.0059 μM in NMDA receptors [183] and a K_b between 1.5 to 3.3 μM in cultured rat brain neurons for AMPA receptors [199]. It is surmised that some of the phasic pain activity may be due to AMPA activity [199]. Another member of this series, ACEA-1328, also has activity at AMPA receptors (K_b of 0.039 μM at NMDA and 3.1 μM at AMPA receptors) [129].

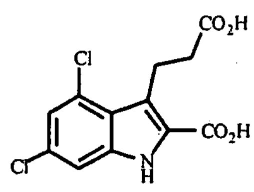
D-Serine is an endogenous co-agonist with a weak reported affinity for [^{3}H] glycine at rat brain membranes of 0.67 μM [186]. However, mice deficient in D-amino-acid-oxidase (DAAO – an enzyme that deoxygenates D-amino acids) have an exaggerated response in late stage formalin. This would suggest that high levels of D-serine could contribute to nociception. The authors suggest that DAAO plays an important neuromodulatory role in regulating D-serine levels [200].

Analogs of D-serine have been explored such as D-cycloserine, a partial agonist at the glycine site. It has a reported intrinsic activity of 57% (*vs.* glycine) in cultured hippocampal neurons [97]. Related to D-cycloserine, R(+)-HA966 is another partial agonist/antagonist at the glycine site, with a reported IC_{50} *vs.* [^{3}H] glycine of 12.5 μM in rat cortical membranes. It was also found to have a K_i = 2.5 μM versus glycine potentiated NMDA responses [188] with an intrinsic activity of 13% in cultured hippocampal neurons [194]. Administration of R(+)-HA966 reduced thermal hyperalgesia before injury and after injury in the CCI rat model (i.th.) [189]. A final related compound, L-687,414, is a low efficacy partial agonist which acts as a functional antagonist at the glycine site [193]. The reported IC_{50} *vs.* [^{3}H] glycine was 1.4 μM in rat cortical membranes. The measured K_i was equal to 0.65 μM *vs.* glycine-potentiated NMDA responses [188]. Reports of pain studies are captured in Table **3**.

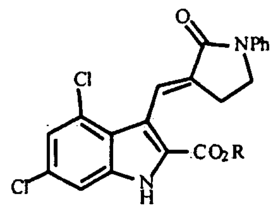
Another class of glycine antagonists evolved from kynurenic acid. Kynurenic acid is a metabolite of tryptophan and may act as an endogenous ligand at the glycine site of the NMDA receptor [201]. It is a weak antagonist with a reported IC_{50} of 41 μM *vs.* glycine in rat cortical membranes [202] and a reported IC_{50} of 7.9 μM *vs.* [^{3}H] MDL 105,519 [194]. Further potency was achieved with the introduction of a 7-chlorine substituent to give 7-chlorokynurenic acid. This compound is a more potent analog of kynurenic acid with a reported IC_{50} of 5.2 μM versus [^{3}H] glycine [202], and an IC_{50} of 0.320 μM [175] for displacement of [^{3}H]L-689,560 from rat cortical membranes. It is reported to have selectivity for NR1A/NR2A over NR1A/NR2B (>10-fold) [88]. Intrathecal administration to rats reduced wind-up after repetitive C-fiber stimulation. It also reduced neuronal responses in the formalin test [203].

DPCQ (or 5,7-DCK = 5,7-dichloro-2,4-dihydroxy-3-phenyl-quinoline dione) is a compound of the same family as 7-CKA. Of interest to the emerging data surrounding peripheral effects of NMDA antagonists, DPCQ was studied as a compound with limited CNS exposure as compared to L-701,324 (a glycine antagonist with higher CNS exposure). Both compounds were active in late-stage formalin, CCI and spinal nerve ligation models, but only L-701,324 worked to block iontophoretic adminstration of NMDA [204]. The conclusion reached by the authors is that peripheral NMDA receptors may play an important role in neuropathic pain mechanisms. L-701,324 is a highly selective and potent antagonist, which is reported to have good oral bioavailability and brain penetration [205].

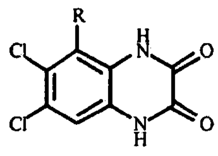
At least two series have been reported which mimic the kynurenic acid portion using a pyridazine-quinolinedione. The quinolinedione portion is acidic and is a suitable mimetic for the carboxylic acid functionality. MRZ 2/576 is reported to be a short-acting compound with rapid entry into the brain but with short duration [194]. A similar compound,



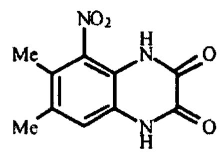
MDL 29,951



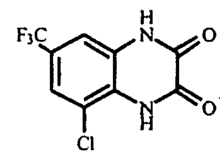
GV-196771, R = H
GV-196771A, R = Na^+



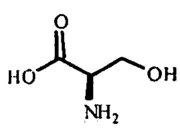
DCQX, R = H
ACEA-1021 (Licostinel)
R = NO_2



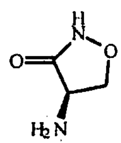
ACEA-1328



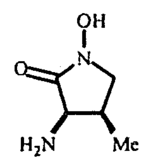
ACEA-1011



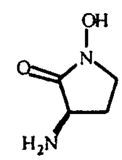
D-serine



D-cycloserine

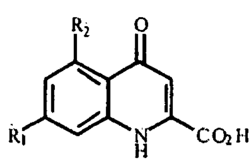


L-687-414

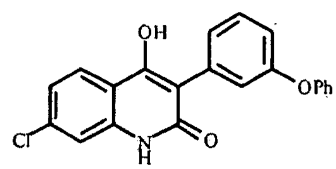


(+)-HA-966

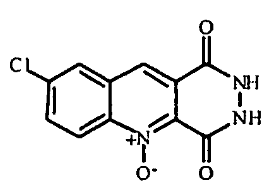
Fig. (5). Glycine-Site Antagonists and Partial Agonists.



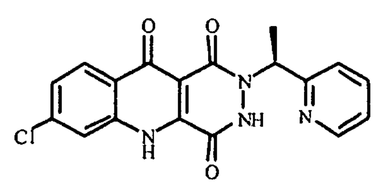
Kynurenic acid, R = H
7-CKA, R1 = Cl
DCPQ or 5,7-DCK R1 = R2 = Cl



MDL 701, 324



MRZ 2/576



AZ-2-PQT

Fig. (6). Glycine-Site Antagonists.

Table 3. Pain Data Associated with Allosteric Glycine Site Antagonists

Antagonist/ *In Vitro* activity	Pain Models	Admin.[a]	Species[a]	Result[b]	Lack Side Effect[b]	Ref.
MDL 29,951 $Ki = 0.14$ µM [177][c]	Tail-flick, heat or pressure	i.p.	m	(-)		[74]
	Formalin, LP	i.p.	m	(+)	(+)	[74]
GV196771A $Ki = 0.027$ µM [178][c]	Formalin, EP	p.o.	m	(-)		[103]
	Formalin, LP	p.o.	m	(+)	(+)[d]	[103]
	Thermal hyperalgesia; CCI	p.o.	r	(+)	(+)[d]	[103]
	Mechanical hyperalgesia; CCI	p.o.	r	(+)	(+)[d]	[103]
	Chronic neuropathic pain	p.o.	h	(-)		[179]
DCQX Kb = 0.38 mM [180]e	Formalin, LP	i. th.	r	(-)		[151]
ACEA-1011 K_b= 0.4 to 0.8 µM [182][e]	Formalin, LP	i.p.	m	(+)	(+)	[181]
	Formalin, EP[e]	i.p.	m	(+)		[77]
	Formalin, LP[e]	i.p.	m	(+/-)[f]		[77]
ACEA-1021 $K_b = 0.0059$ µM [183][e]	Tail flick	i. th.	m	(+)		[129]
	Formalin	i.th.	m	(+)		[129]
	Formalin, opiate attenuation	i.th.	r	(+)	(-)[g]	[184]
ACEA-1328 $K_b = 0.039$ µM [129][e]	Tail flick	i. th.	m	(+)		[129]
	Tail flick, κ-opiod attenuation	i. p.	m	(+)		[185]
D-serine $IC_{50} = 0.354$ µM [186][c]	Formalin, κ,µ-opiod attenuation	i.c.v.	r	(+)		[187]
D-cycloserine $IC_{50} = 7.37$ µM [186][c]	Tail flick	s.c.	m	(-)		[74]
	Formalin, EP	s.c.	m	(-)		[74]
	Formalin, LP	s.c.	m	(+)	(+)[h]	[74]
R(+)-HA-966 $IC_{50} = 12.5$ µM [188][c]	Tail flick	i. th.	m	(-)		[74]
	Formalin, EP	s.c.	m	(-)		[74]
	Formalin, EP	s.c.	r	(-)		[156]
	Formalin, LP	s.c.	m	(+)	(+)[h]	[74]
	Formalin. LP	s.c.	r	(+)	(+)[h]	[156]
	Thermal hyperalgesia, CCI	i.th.	r	(+)		[189]
	Mechanical allodynia, opiod attenuation	s.c	r	(+)	(+)	[190]
	Mechanical allodynia, nerve injury- opiate attenuation	s.c.	r	(+)	(+)[h]	[191]
	Themmal allodynia, nerve injury - opiate attenuation	s.c.	r	(+)	(+)[h]	[191]
	Formalin, EP- attenuation of NK1 antagonist	s.c.	m	(+)	(+)[i]	[192]
L-687,414 $IC_{50} = 1.4$ µM[188][c]	Tail flick	s.c.	m	(-)		[74]
	Formalin, EP	s.c.	m	(-)		[74]
	Formalin, LP	s.c.	r	(+)	(+)[h]	[74]
	Mechanical hyperalgesia, carrageenan	i.p.		(+)	(-)[j]	[193]
	Mechanical allodynia, nerve injury	i.p.	r	(+)	(+)[k]	[141]

(Table 3) Contd….

Antagonist/ *In Vitro* activity	Pain Models	Admin.[a]	Species[a]	Result[b]	Lack Side Effect[b]	Ref.
Kynurenic acid IC_{50} = 7.9 μM [194][l]	Thermal hyperalgesia, CCI	i.th.	r	(-)		[126]
	Thermal hyperalgesia, carrageenan	i.th.	r	(+)	(-)	[195]
	Thermal hyperalgesia, carrageenan--μ-opiod attenuation	i.th.	r	(+)	(+)	[195]
7-CKA IC_{50} = 0.32 μM [175][m]	Thermal hyperalgesia, carrageenan	i.th.	r	(+)	(+)	[85]
	Formalin, LP	i.th.	r	(-)		[151]
DPCQ or 5,7-DCK IC_{50} = 0.064 μM [175][m]	Formalin, EP	i.p.	m	(-)		[74]
	Formalin, LP	i.p	m	(+)		[74]
	Tail-flick, heat or pressure	i.p.	m	(-)		[74]
L-701,324 IC_{50} = 0.002 μM [193][m]	Mechanical hyperalgesia, carrageenan	i.p.		(+)	(+)	[193]
	Mechanical hyperalgesia, carrageenan	i.p.	r	(+)	(-)	[141]
	Mechanical allodynia, nerve injury	i.p.	r	(+)	(-)	[141]
	Thermal hypersensitivity, PGE2	i.p.	r	(+)		[121]
MRZ 2/576 IC_{50} = 0.100 μM [194][l]	Tail flick, opiate attenuation	i. p.	m	(+)[j]		[196]
	Uterer distension, reflex pressor response	i.v.	r	(+)		[120]
	Toe pinch	i.v.	r	(-)		[120]
AZ-2-PQT *Ki* = 0.207 μM [197][l]	Thermal hyperalgesia, CCI	p.o.	r	(+)	(+)[k]	[197][n]
	Formalin, LP	p.o.	r	(+)	(+)[k]	[197][n]

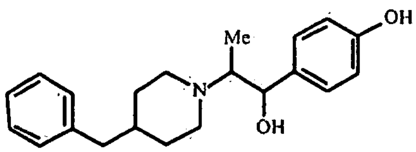
[a] Route of administration and species tested: i.th. = intrathecal; i.p. = intraperitoneal, i.m. = intramuscular, i. pl. = intraplantar, s.c. = subcutaneously, i.v. = intravenously, p.o. = oral,; r = rat, m = mouse, h = human. [b] Result : (+) = postive result, (-) = negative result, (+/-) = limited efficacy or incomplete attenuation. Side effect separation (+) = no noticeable effects in assay, (-) = noticeable effects, no value = not reported. [c] Versus [^{3}H] glycine. [d] Based on references cited therein. [e] Affinity against expressed receptors in Xenopus oocytes. [f] HA and LA MICE, in late-phase formalin the activity was seen in HA but not LA mice. [g] Side effects seen at maximal useable dose. [h] Therapuetic margin observed between *in vivo* pain assay and motor function assay. [i] Did not enhance the ataxia of NK1 antagonist. [j] Mild ataxia seen at MED. [k] Authors report only a narrow window versus side effects. [l] Versus [^{3}H] MDL 105,519. [m] Versus [^{3}H] L-689, 560. [n] *In vitro* data is reported in reference [197]. Pain data is unpublished, but previously reported: Brown, D. G.; Bare, T. M.; Urbanek, R. A.; McLaren, F. M.; Horchler, C. L.; Murphy, M.; Steelman, G. B.; Empfield, J. R.; Forst, J. M.; Herzog, K. J.; Xiao, W.; Dyroff, M. C.; Lee, C. M. C.; Trivedi, S.; Neilson, K. L.; Keith, R. A. 7-Chloro-2,3-Dihydro-2-[1-(pyridinyl)alkyl]-pyridazino[4,5-b]quinoline-1,4,10(5H)-triones as NMDA Glycine-Site Antagonists with Antinociceptive Activity. *Abstracts of Papers, MEDI-169, 227th ACS National Meeting*, Anaheim, CA, United States, March 28-April 1, 2004.

AZ-2-PQT, is a potent and selective full antagonist at the glycine site [197]. The compound demonstrated good oral bioavailability and oral activity in two distinct pain models. Pain data from these compounds are reported in Table 3.

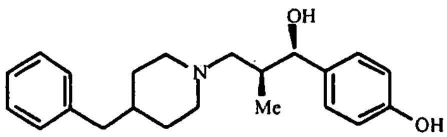
POLYAMINE SITE/IFENPRODIL SITE ANTAGONISTS

Both spermine and spermidine are known to modulate activity at the NMDA receptor. Polyamines of this type tend to have biphasic responses. Depending on the concentration and the structure of the polyamine, they may exert both agonist and antagonist modulation [206]. It has been reported that ifenprodil and other compounds of similar nature are non-competitive antagonists of the polyamine site [207]. Polyamine site antagonists related to ifenprodil tend to be selective for the NR2B subtype. This is observed within the heterogeneous assembly of the various subtypes, as different constructs of NR1/NR2A-D possess differing responses to polyamines. The NR2B subtype has been studied due to the potential for fewer and milder side-effects as compared to those associated with competitive antagonists and channel blockers. This is hypothesized based on the distribution of NR2B receptors as compared to other subtypes. In rat brain, NR2B is exclusively located in the forebrain and not the cerebellum [208]. It is also found to be located predominately in the dorsal horn of the spinal cord [141]. It is important to note that this field remains an area of active research and, as of yet, a clear understanding of the roles of subtype populations in neuropathic pain events has not been established. It is apparent however, that NR2B subtype selective antagonists do appear to have fewer side effects than competitive and non-competitive antagonists in animal models of neuropathic pain. It should also be noted that mice over-expressing NR2B receptors in the forebrain show greater mechanical allodynia after formalin or CFA compared to wild type. No changes were observed for acute pain [209]. For a review of NR2B pharmacology see Loftis [210].

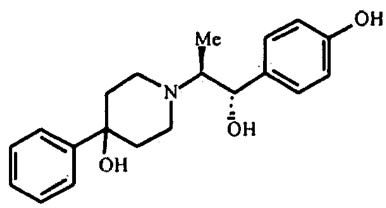
Ifenprodil is a potent NR2B selective antagonist that binds at the polyamine site [211, 212]. Ifenprodil demons-



Ifenprodil



Ro25-6981



CP-101,606

Fig. (7). Polyamine/Ifenprodil Site Antagonists

trated activity in carrageenan-induced mechanical nociception (i.p., rats), however did not demonstrate NMDA antagonism in spinal cord using electrophysiological studies (i.v.). These results suggest a supraspinal site of action [64]. (+/-)Ro 25-6981 is a close analog to ifenprodil, and is reported to have measured antinociceptive activity (Table **4**).

Similar in structure to both ifenprodil and Ro 25-6981, CP-101,606 has also been studied in pain models. Even though it is a compound with a relatively short half-life ($t_{1/2}$ = 20 min rat, s.c), it has the ability to distribute into the brain and spinal cord [213]. It has been observed that the site of action of CP-101,606 is most likely in the brain and not in spinal locations. This was concluded based on anti-hyperalgesia from intracerebroventricular administration (CCI model) and the lack of an anti-hyperalgesia effect when administered intrathecally [214]. Similar to the glycine-site antagonists, reports have emerged which suggest an acceptable therapeutic window for this class of compounds [141].

ALKYL GUANIDINES

Several alkyl guanidine compounds have been reported to have NMDA binding activity and antinociceptive activity (Fig. **8**). These compounds are complex to understand, since they tend to have substantial ancillary pharmacology. However a summary of pain related activities in context to NMDA receptor binding is included.

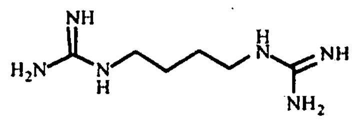
Arcaine sulfate (ARCA) caused hyperalgesia in thermal flexion reflex tests. It was ineffective in formalin and mechanical flexion reflex test but did not cause motor dysfunction at the highest dose studied [151]. Similar to agmatine, it is proposed to bind the open channel [215, 216].

Agmatine is reported to be an endogenous antagonist of NMDA receptors, however it has activity associated with other receptors such as imidazoline and α_2-adrenergic receptors [217]. Agmatine is suggested to be acting *via* an open channel block, and is competitive with MK-801 [216]. Other studies link agmatine to the polyamine site. The K_i for spermine-potentiated [^{3}H] MK-801 binding is 14.8 μM compared to > 500 μM for direct displacement of [^{3}H] MK-

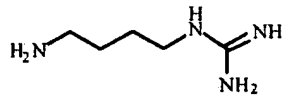
Table 4. Pain Data Associated with Polyamine Site/Ifenprodil Site Antagonists

Antagonist/ *In Vitro* activity	Pain Models	Admin.[a]	Species[a]	Result[b]	Lack Side Effect[b]	Ref.
Ifenprodil IC_{50} = 0.250 μM @NR2B; >40 μM @NR2A [81]	Mechanical nociception, carrageenan	i.p.	r	(+)		[64]
	Mechanical allodynia, nerve injury	i.p.	r	(+)	(-)	[141]
	Thermal hypersensitivity, PGE2	i.p.	r	(-)		[212]
(+/-)Ro 25-6981 IC_{50} = 0.009 μM @NR2B; > 60 μM @ NR2A [81]	Mechanical allodynia, nerve injury	i.p.	r	(+)	(+)	[141]
(+/-)-CP-101,606 IC_{50} = 0.060 μM @NR2B; >100 μM @NR2A [81]	Mechanical hyperalgesia, carrageenan	s.c	r	(+)	(+)[c]	[213]
	Capsaicin-induced nociception	s.c	r	(+)	(+)[c]	[213]
	PMA-induced nociception	s.c	r	(+)	(+)[c]	[213]
	Mechanical allodynia, nerve injury	p.o.	r	(+)	(+)	[141]
	Mechanical hyperalgesia, carrageenan	p.o	r	(+)	(+)	[141]

[a] Route of administration and species tested: i.p. = intraperitoneal,; r = rat. [b] Result : (+) = postive result, (-) = negative result. Side effect separation (+) = no noticeable effects in assay, (-) = noticeable effects, no value = not reported.[c] Therapeutic margin observed between *in vivo* pain assay and motor function assay.



ARCA
Arcaine



Agmatine

Fig. (8). Alkyl guanidines.

801 [218]. It possesses activity (i.p.) in early phase and late phase formalin. However, the activity of agmatine in late phase formalin but not early phase can be blocked by yohimbine suggesting a role of α2 receptors. There was no reported activity in tail flick model in this same study [219]. Agmatine is known to be an allosteric modulator of α2A receptors (~10 μM), which can mediate nociception as well [220]. A recent study has suggested that (*i.th.*) antinociceptive effect of agmatine in a tail pinch model is related to the imidazoline receptors [221]. Agmatine has shown efficacy in a carrageenan-evoked mechanical hyperalgesia, dynorphin-induced allodynia and nerve-injury models (Chung model, *i.th.*, admin.) with no effect on behavior changes. There was no effect in acute pain tests. The authors suggest that agmatine may play an endogenous role in regulating neuropathic pain [222].

CONCLUSION

NMDA antagonists are well-studied in a variety of neuropathic pain models, and results suggest they may be useful for treating the pathological conditions underlying neuropathic pain while not affecting the normal physiological pain responses. However, deleterious side-effects observed with many of the compounds have raised the question if this is a mechanism-based effect which cannot be overcome. It appears that within the non-competitive class of NMDA receptor antagonists, the most potent compounds (*e.g.* MK-801) are unsuitable for clinical use due to the side effect profile. Low affinity non-competitive antagonists may be more useful, with fewer observable side effects. For example, memantine has demonstrated a superior side-effect profile, and is currently in use for other diseases. However, memantine did not show efficacy in several models of clinical pain.

Advances in the competitive NMDA class of compounds have led to the discovery of the peptides Con-G and Con-T which represent NR2B subtype selective competitive antagonists with the potential for improved side-effect profiles. However, the peptidic nature of these compounds would certainly diminish the chances of these compounds being orally efficacious treatments for pain. The glycine site-antagonists also represent a potential area for future pain therapies. Many of the initial glycine-site antagonists suffered from poor physicochemical properties that may preclude future clinical development and may have complicated the *in vivo* assays. More recently, orally efficacious glycine-site antagonists have been discovered that demonstrate good efficacy in animal models of neuropathic pain. Ongoing clinical trials with NMDA antagonists in neuropathic pain will help further establish if these types of compounds are useful in clinical practice. Finally, emerging literature on the role of peripheral NMDA receptors in neuropathic pain may lead to a better understanding of how CNS-mediated side effects of NMDA-antagonists might be avoided.

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ABBREVIATIONS

FCA	=	Freund's complete adjuvant.
CCI	=	Chronic constrictive nerve injury
BBB	=	Blood-brain barrier
EP	=	Early phase (formalin assay)
LP	=	Late Phase (formalin assay)

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